Binding of DU145 Prostate Tumor Cancer Cells to Silicon Carbide

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Introduction:
Prostate cancer occurs in men worldwide. The risk factors vary among different ethnic groups. Prostate cancer has been detected in European and American men more so than in East and South Asian men [1]. DU145, one of the three “classical” cell lines of prostatic cancer, was isolated by K.R. Stone et al. from a wound in the brain of a patient with metastatic carcinoma of the prostate and a three year history of lymphocytic leukemia. The cell line is not detectably hormone sensitive and does not express prostate specific antigen (PSA) [2].

The long-term goal of this study is to explore an alternative technique to differentiate biological cells on silicon carbide substrates by their electrical properties using scanning tunneling microscopy (STM) and a probe technique. To this extent, the specific objective of this project was to examine the binding of metastatic DU145 prostate cancer cells to different types of silicon carbide (SiC): 3C-SiC grown on Si, 6H-SiC, and highly doped (HD) 6H-SiC to determine the most effective binding substrate.

SiC is biocompatible and is utilized in many biomedical applications such as stents, orthopedic implants, in drug delivery and tissue engineering [3]. For applications such as tissue engineering it is important to examine the binding potential and proliferative capabilities of cells as they develop into tissue on SiC substrates. The 3C, 6H and HD 6H poly types of silicon carbide were considered because of their electrical conductivity between that of metals and insulating materials [4]. Depending on their crystal structure and if the material is doped, then poly types have high thermal conductivity and high electron mobility that may be useful in a bio-electronic device.

The number 3 in 3C refers to the three-bilayer periodicity of the stacking structure and the letter C denotes the cubic symmetry of the crystal; similarly, the number 6 in 6H refers to the six-bilayer periodicity of the stacking structure and H denotes the hexagonal symmetry of the crystal.

Experimental Procedure:

Substrate Preparation. The substrates shown in Figure 1 were cleaned by ultrasonic bath using soap, water, acetone, and methanol sequentially for three minutes each. Cleaned substrates were placed in a dust free cloth lined container.

Tumor Cell. DU145 was purchased from American Type Culture Collection (ATCC). The cells were cultured in complete Roswell Park Memorial Institute (RPMI) 1640 media {5% fetal bovine serum, gentamycin (50mg/ml), penicillin/ streptomycin (5,000 units / 5,000 mg/ml)} in a humidified atmosphere of 5% CO₂ and 95% air.

Binding of Cells to SiC Substrates. DU145 cells were harvested, either from a frozen sample by quick thaw or from trypsin-treated cultures. The cells were counted and adjusted to various concentrations. Substrates were placed into the wells of a 6-well culture plate; 2 ml of cells were added to each well. The plate was incubated overnight at 37°C in 5% CO₂. An Olympus IX71 inverted microscope with an Olympus DP70 camera was used to confirm binding and sizes of the cells on the substrate. Optical microscope images of the cells bound to 6H and HD 6H-SiC are shown in Figure 2.
Fixing of DU145 Tumor Cells to SiC Substrates. Substrates with DU145 cells were placed into individual wells of a six well plate; 2 ml of methanol was added to each well. After five minutes, the substrates were bathed in water. Five minutes later, the substrates were placed in an empty well and allowed to dry. Atomic force microscopy (AFM) was used to obtain three-dimensional high resolution images of the cells [5].

Experimental Design. Three different experiments (trials) were executed with various concentrations of DU145 cells to determine: 1) the optimal cell concentration for the examination of individual cells, and 2) the most effective substrate for binding cells.

Results and Conclusions:
A procedure for preparing samples that would allow for the discrimination of cells in a heterogeneous mixture of cells that could be used in STM and the probe technique was developed using the DU145 cell line. Luminal-like cells with a width and length of 44.091 µm and 6.452 µm, respectively, were observed on 6H-SiC and HD 6H-SiC while binding on 3C-SiC was undetermined because of its lack of transparency. The optimum concentration of cells was determined to be $5 \times 10^4$ cells/ml. Highly doped 6H-SiC was the better substrate for observing the cells’ morphologically.

Future Work:
We plan to apply STM and the probe technique to measure electrical properties (i.e. current-voltage curves) of the cells as an alternative method to differentiate biological materials.

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